Species, Vol. 17, No. 56, July 1, 2016 REPORT

ISSN 2319–5746 EISSN 2319–5754

Species
An International Journal

Morphological description of *Myxobolus markiwi* n. sp. (Cnidaria: Myxosporea: Myxozoa) infecting gills of fingerlings of aquaculture ponds from Punjab, India

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Publication History
Received: 14 August 2016
Accepted: 1 September 2016
Online First: 6 September 2016
Published: July-September 2016

Citation
Harpreet Kaur, Ishtiyaq Ahmad. Morphological description of *Myxobolus markiwi* n. sp. (Cnidaria: Myxosporea: Myxozoa) infecting gills of fingerlings of aquaculture ponds from Punjab, India. *Species*, 2016, 17(56), 141-149

General Note
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ABSTRACT

During a survey from November 2014 to October 2015, 50 fingerlings of *Labeo rohita* (Hamilton) were collected from the village Fagan Majra, District Fatehgarh Sahib, Punjab, out of which gills of 20 fishes were found to be infected with a new myxozoan parasite belonging to the genus *Myxobolus* viz. *M. markiwi* n. sp. The total prevalence of infection rate was recorded as 40%. The length and age of the fish host was 3.8cm and 1-2 months respectively. The plasmodia were 0.2mm in diameter were seen as

creamish pustules on the gills. The myxospores were oval in shape with a prominent knob at an anterior end and measured 6.54x5.35µm. Polar capsules were equal, pyriform, measure 1.87x0.86µm with narrower anterior end attached to the knob and rounded posterior end. The gill plasmodial index (GPI) was recorded to be 1 indicated light infection. Polar filament coils 4-5 in number, arranged obliquely to the polar capsule axis. Intercapsular process (ICP) absent. Sporoplasm agranular, homogenous, with two nuclei, 0.2-0.3µm in diameter. Iodinophilous vacuole absent.

Key words: Fingerlings, *Myxobolus markiwi*, GPI, Prevalence

1. INTRODUCTION

Aquaculture development in Punjab is increasing day by day, representing it as the fastest growing animal husbandry. Punjab government is paying a lot of attention to the fisheries sector and investment in this field has been increasing all through the plan periods (Vasisht and Singh, 2009). The area under fish culture is 10856.6 ha. The Fagan Majra fish farm is located in the district of Fatehgarh Sahib managed by Fisheries Department, Government of Punjab consists of a total of 59 culture ponds. Out of which 14 are hatchery/ nursery ponds in which Indian major carps such as catla, rohu, mrigal, common carp, grass carp and silver carp are cultured in a semi-intensive polyculture system for supply all over the state of Punjab and adjoining areas.

Classification of the Phylum Myxozoa have a long history since the discovery of Myxosporea by Jurine (1825) and later described by Muller (1841). Myxozoans are microscopic eukaryotic organisms, obligate parasites of vertebrates and invertebrates with extremely reduced body size, structure and possess very complicated life cycles characterized by the formation of myxospores (Morris and Adams, 2007). Myxosporeans were thought to be protisists for more than one hundred years until the 1900s due to the simpilicity of their microscopic myxospores.

More recent phylogenetic analysis (Jimenez-Guri *et al*., 2007) based on the sequences of numerous protein-coding genes of the malacosporean, *Buddenbrockia plumatellae* have suggested Cnidaria as the most closely related taxon to Myxozoa. Shpirer *et al*. (2014) based on phylogenomics of 4 nematogalectin genes have placed them within Cnidaria as the members evolved very early and becoming parasitic due to simple body organization.

Myxozoans are common parasites of fishes but a few species infect other vertebrate hosts such as amphibians, reptiles, waterfowl etc. Great economic losses caused by these parasites in aquaculture have been well documented (Lom and Dykova, 2006). Due to pathogenic potentials of some species they can affect reproduction, growth and involve epizooties being able to cause the death of the host (Longshaw et al., 2005). More recently in Punjab (India), many species have been described infecting freshwater fishes in wetlands and aquaculture (Singh and Kaur, 2012a, b, c, d; Singh and Kaur, 2014; Kaur, 2014; Kaur et al., 2014a, b; Kaur and Katoch, 2014; Singh and Kaur, 2015: Kaur and Gupta, 2015; Kaur and Katoch, 2016). Basu et al. (2015) gave a summarized compilation of 32 species in India. So in order to increase efficiency, it is important to identify the root cause where management and preventions can assist farmers in reducing risk factors and increasing the commercial value of the fish product. Keeping in view the lack of information in the myxozoan species infecting fingerling, the present study was carried out in local nursery ponds.

2. MATERIALS AND METHODS

Live fingerlings were collected from the village Fagan Majra, District Fatehgarh Sahib, Punjab. Fishes were brought to the laboratory and examined for the presence of myxozoan infection. Various organs like scales, gills, fins, intestine, and kidney were examined but only gills were found infected. Plasmodium from gills of infected *Labeo rohita* (Ham.) were smeared on clean slides in a drop of 0.98% Nacl solution covered with coverslip and were examined for the presence of spores. Fresh myxospores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent prepration, air-dried smears fixed in Bouin's fixative were stained with Ziehl-Neelsen and Iron-haematoxylin. Line Drawings were made from stained material with the aid of camera lucida (Fig. 3a-c). Measurements of myxospores were done with the aid of calibrated ocular micrometer. The gill plasmodial index (GPI) was calculated on the basis of number of plasmodia present per gill on one side visible under the sterozoom binocular microscope and with the naked eye (Kaur and Attri, 2015) 0-0 (no infection-0); 1-5 (light infection-1); 5-10 (moderate infection-2); 10-20 (heavy infection-3); 20-50 or more (severe infection-4). Categorization of plasmodia on the basis of size (Kaur and Katoch, 2016).

Type A: Plasmodia visible under binocular microscope (size range = 40-200µm)

Type B: Plasmodia visible under stereozoom (size range = 0.2-0.9mm)

Type C: Plasmodia visible with naked eye (size range = 0.9-3mm)

For prevalence, following formula was used

$$2.1\ \text{Prevalence}\ (\%) = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \times 100$$

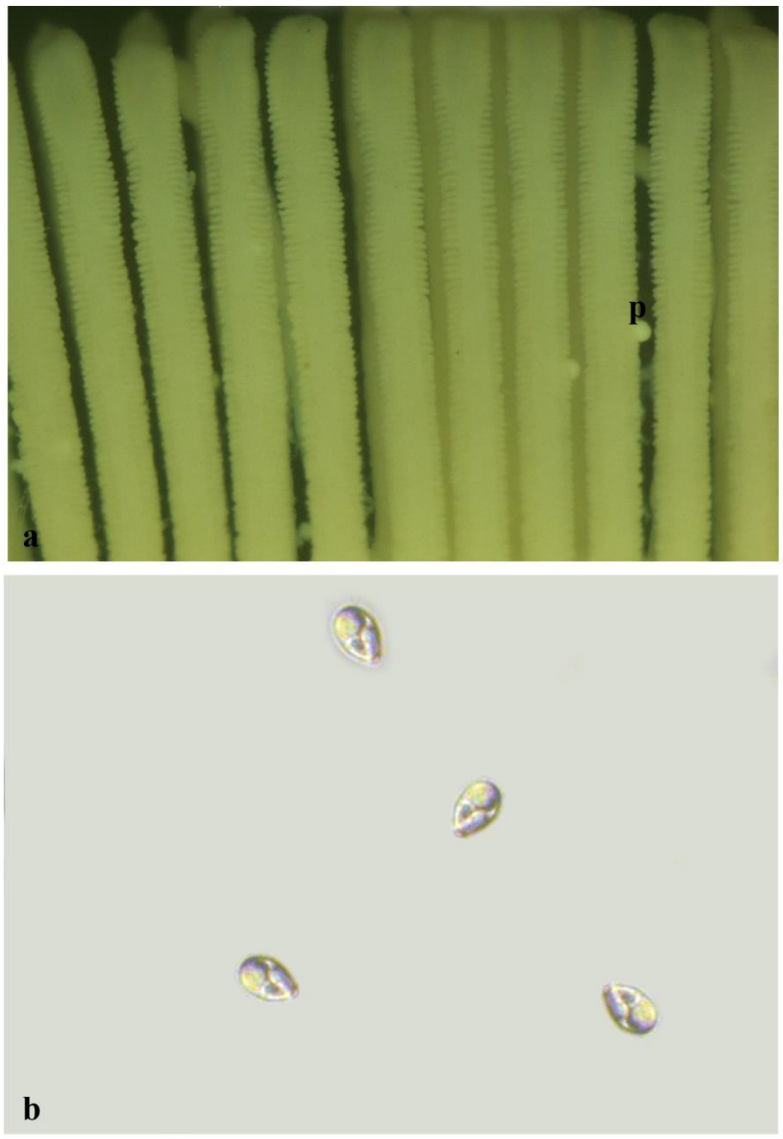

Fig. 1

Figure 1

(a) Gills of *Labeo rohita* showing plasmodia of *M. markiwi* n. sp. under stereozoom microscope

(b) Myxospores of *M. markiwi* n. sp. in fresh preparation (phase contrast) in frontal view (Scale bar 20µm)

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3. RESULTS

Myxobolus markiwi n. sp.

Plasmodia (Fig. 1a,b)

Minute, microscopic, round to oval, creamish, measure 0.2mm in diameter, attached to gill lamellae, histozoic, 2-4 in number per gill, 20-30 myxospores present per plasmodium. No clinical signs.

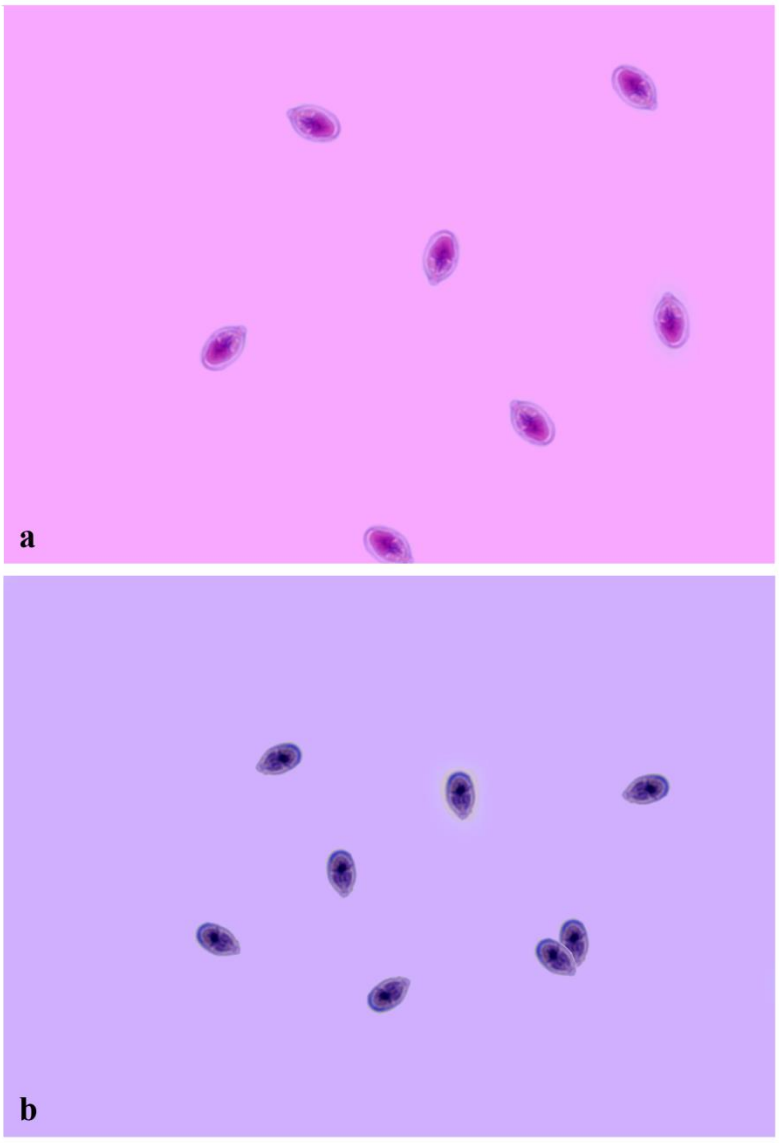

Fig. 2

Figure 2

(a) Myxospores of *M. markiwi* n. sp. stained in Ziehl-Neelsen (Scale bar 20µm)

(b) Myxospores of *M. markiwi* n. sp. stained in Iron haematoxylin (Scale bar 20µm)

Myxosopore description (Fig. 2a,b;Table 1)

(Measurements based on 10-12 myxosopores in frontal view)

Myxospores measure 6.54x5.35µm, ovoidal in frontal view, anterior extremity more slender than posterior extremity, bluntly pointed anterior end with prominent knob and rounded posterior end. Sutural line straight. Both shell valves smooth, thin, symmetrical,

measure 0.20µm thick. Parietal folds absent. Polar capsules two, equal, pyriform, measure 1.87x0.86µm, with narrower anterior end attached to the knob and rounded posterior end, occupying less than half of the myxospore body cavity. Polar filament coils 4-5 in number, arranged obliquely to the polar capsule axis. Intercapsular process (ICP) absent. Sporoplasm agranular, homogenous, with two nuclei, 0.2-0.3µm in diameter. Iodinophilous vacuole absent.

Table 1 Measurments (µm) and ratio of *M. markiwi* n. sp.

Characters	Range	Mean Values	SD
LS	5.60-7.48	6.54	0.25
WS	4.35-6.35	5.35	0.46
LPC	1.25-2.49	1.87	0.22
WPC	0.36-1.36	0.86	0.46
Ratio: LS/WS		1.22	
ICP		Absent	
NC		4-5	
Parietal Folds		Absent	

Taxanomic summary of *M. markiwi* n. sp.

Family : Myxobolidae
Type host : *Labeo rohita* Hamilton vern. rohu
Family: Cyprinidae
Age of the fish host : 1-2 months
Length of the fish : 3.8 cm
Type locality : Nursery Pond, Fagan Majra, District Fatehgarh Sahib, Punjab (India)
Type specimen : Paratypes are myxospores stained in Ziehl-Neelsen and Iron-haemotoxylin, deposited in the Parasitology Laboratory, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India. Slide no. LR/ZN/21.01.2015 and HM/IH/21.01.2015
Site of infection : Gill lamellae (Intralamellar vascular type LV1)
Type of plasmodia : Type A (visible under binocular microscope)
Prevalence of infection (%) : 40% (20/50)
Gill plasmodial index (GPI) : 1 (2-4 plasmodia per gill) indicating light infection
Clinical symptomatology : No clinical signs
Etymology : The specific epithet '*markiwi*' is after the name of Dr. M. E. Markiw for his novel study on the life cycle of the phylum Myxozoa

4. DISCUSSION

The present species is compared with morphologically similar myxospores of the species i.e; *M. batae* Karamchandani (1970) infecting gill filament of *Labeo bata* Hamilton; *M. bankimi* Sarkar (1999) infecting inner wall of gall bladder of *Sicamugil cascasia* Hamilton; *M. bhadurius* Sarkar (1985) infecting gall bladder of *Wallago attu*; *M. coeli* Haldar *et al.* (1996) infecting gall bladder of *Chanos chanos*; *M. filamentosus* Haldar *et al.* (1985) infecting cartilage and brain of *Puntius filamentosa*; *M. hyderabadense* Lalitha Kumari (1969) infecting gill filament of *Barbus pinnauratus*; *M. indiae* Lalitha Kumari (1969) infecting gill filament of *Barbus sarana*; *M. macrolepi* Padma *et al.* (1992) infecting intestine of *Liza macrolepis*; *M. meglitschus* Sarkar (1996) infecting epithelium of gills of *Notopterus notopterus*; *M. molae* Sarkar (1993) infecting kidney of *Amblypharyngodon mola*; *M. mrigalae* Chakravarty (1939) infecting scales of *C. mrigala*; *M. mrigalhitae* Basu and Haldar (2003) infecting gills of *C. mrigala* and *L. rohita*; *M. mystusius* Sarkar (1986) infecting scales of *Mystus vittatus*; *M. narasii* Narasimhamurti (1970) infecting intestine of *Mugil waigensis*; *M. orissae* Haldar *et al.*

(1997) infecting gills of *C. mrigala* Hamilton; *M. potaili* Lalitha Kumari (1969) infecting liver and intestine of *L. potail* and *M. potularis* Madhavan, Bandyopadhyay and Santosh (2013) infecting gill filaments of *L. calbasu* Hamilton, *L. bata* Hamilton, *L. gonius* and *C. reba* but differed from all of the above in morphological and morphometrical characteristics (Table 2).

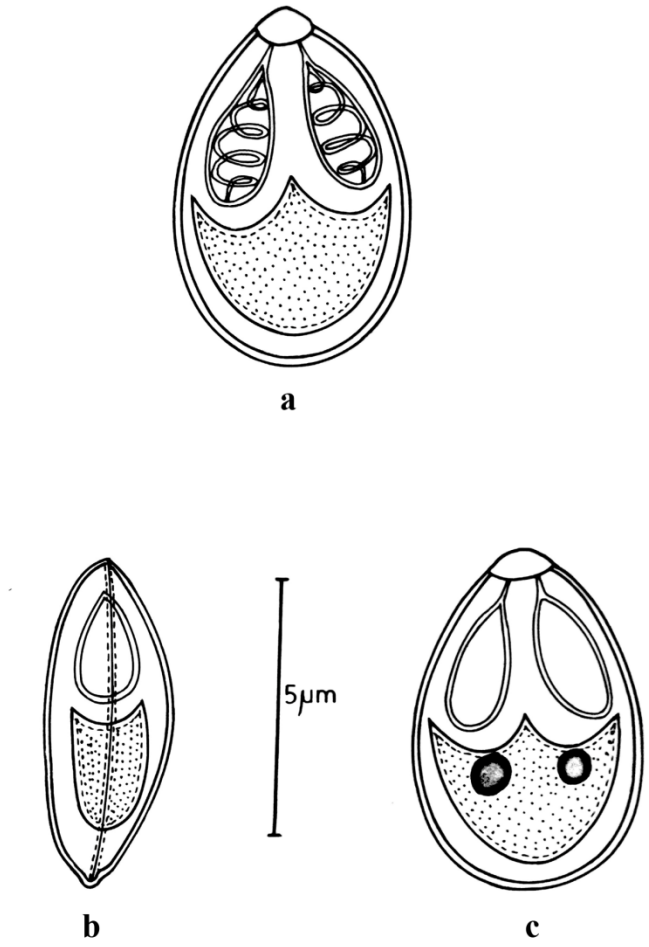

Fig. 3

Figure 3

(a) Line drawing of fresh mature myxospore of *M. markiwi* n. sp. stained in Ziehl-Neelsen (Frontal view).

(b) Line drawing of fresh mature myxospore of *M. markiwi* n. sp. (Sutural view)

(c) Line drawing of fresh mature myxospore of *M. markiwi* n. sp. stained in Iron haematoxylin (Scale bar = 5µm)

Table 2 Comparative description of *M. markiwi* n. sp. with morphologically similar species (measurements in micrometer)

Species	Host	Site of infection	Locality	Myxospore	Polar capsule
***M. markiwi* n.sp. (Present study)**	*Labeo rohita*	Gill lamellae	Nursery Pond, Fagan Majra, Punjab (India)	6.54x5.35	1.87x0.86
M. potaili Lalitha	*Labio potail*	Liver,	Andhra Pradesh	7.2x5.4	3.3x2.0

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Kumari,1969		Intestine	(India)		
M. potularis Madhavan, Bandyopadhav and Santosh, 2013	*L. calbasu, L. bata, L.gonius, Cirrhinus reba*	Gill filament	West Bengal (India)	9.0x6.0	5.4x2.2
M. mrigalae Chakravarty,1939	*Cirrhinus. mrigala*	Scales	West Bengal (India)	7.2x8.2	5.15x2.09(L) 3.09x2.06(S)
M. indiae Lalitha Kumari,1969	*Barbus sarana*	Gill filament	Andhra Pradesh (India)	13.7x7.3	5.9x2.1(L) 5.2x2.1(S)
M. hyderabadense Lalitha Kumari, 1969	*Barbus pinnauratus*	Gill filament	Andhra Pradesh (India)	10.1x5.9	5.8x2.2
M. batae Karamchandani, 1970	*L. bata*	Gill filament	Orissa (India)	10.4x8.0	4.4x1.6
M. narasii Narasimhamurti, 1970	*Mugil waigensis*	Intestine	Andhra Pradesh (India)	12.8x9.0	3.2x1.7
M. mathuri Jayasri, Parvateesam and Mathur, 1981	*Puntius sarana*	Gills	Rajasthan (India)	16.1x7.6	5.25x3.2
M. filamentosus Haldar, Mukherji and Kundu, 1985	*Puntius filamentosa*	Cartilage and Brain	West Bengal (India)	13.7x9.5	3.6x3.1
M. bhadurius Sarkar,1985	*Wallago attu*	Gall bladder	West Bengal (India)	10.59x6.28	5.31x2.78
M. mystusius Sarkar,1986	*Mystus vittatus*	Scale	West Bengal (India)	13.18x9.39	7.12x3.59(L) 4.05x1.33(S)
M. macrolepi Padma *et al.*,1992	*Liza macrolepis*	Intestine	Andhra Pradesh (India)	6.28x5.26	2.78x2.0
M. molae Sarkar, 1993	*Amblypharyngodon mola*	Kidney	West Bengal (India)	9.00x7.4	6.00x1.55
M. coeli Haldar *et al.*,1996	*Chanos chanos*	Gall bladder	Orissa (India)	10.6x5.49	5.76x2.58(L) 4.2x2.9(S)
M. meglitschus Sarkar,1996	*Notopterus notopterus*	Epitheliu m of gills	West Bengal (India)	9.0x7.4	6.0x1.55
M. orissae Haldar *et al.*,1997	*C. mrigala*	Gills	Orissa (India)	15.71x6.8	8.8x1.78(L) 7.58x2.57(S)
M. bankimi Sarkar,1999	*Sciamugil cascasia*	Inner wall of gall bladder	West Bengal (India)	10.6x8.7	3.97x2.72
M. mrigalhitae Basu and Haldar, 2003	*C. mrigala L. rohita*	Gills	West Bengal (India)	10.8x7.9	4.8x2.1(L) 3.0x2.1(S)

The myxospores of *M. batae, M. meglitschus, M. molae* and *M. orissae* differ in having a prominent intercapsular process (ICP). Furthermore *M. coeli, M. filamentosus*, *M. indiae, M. mathuri, M. mrigalae, M. mrigalhitae, M. mystusius* and *M. orissae* differ from the present species in having unequal polar capsules.

The present species is characterized in having more slender myxospore body with a prominent knob-like structure at the anterior end, pyriform polar capsules with narrower anterior end. In this respect, it is comparable to *M. bankimi, M. bhadurius* and *M. narasii,* however knob is absent in the above mentioned species, in contrast to this a prominent knob in the present species. Furthermore,

M. hyderabadense, *M. indiae, M. macrolepi* and *M. mrigalhitae* also differ in having parietal folds. The present species is comparable to *M. potaili* and *M. potularis* in having a knob at the anterior end. It differ from *M. potaili* in having intercapsular process (ICP) at the anterior end and from *M. potularis* in having more slender myxospore i.e; (LS/WS ratio 1.22 vs 1.63) and smaller polar capsules (1.87x0.86 vs 4.00x1.23).

M. markiwi n. sp. is distinguished from other two new species (having similar shape) i.e. *M. knobii* n.sp. and *M. majraiensis* n. sp. The present species is characterized in having ovoidal myxospore with its extremity more slender, therefore differ from *M. knobii* n. sp. in which the myxospores are oval to spherical and *M. majraiensis* n. sp. in which myxospores are egg-shaped.

In view of the above differences the present species has been proposed as new to science and named as *M. markiwi* n. sp.

CONFLICT OF INTEREST

There is no conflict of interest to disclose.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities provided by Instrumentation lab Punjabi University, Patiala. Financial support for this study was provided by CAS-DST grant Department of Zoology, Panjab University, Chandigarh.

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